

Cell Type Prediction for Intestine Tissue Samples from Brightfield Histology via Deep Learning

Sayat Mimar^{a†}, Nicholas Lucarelli^{a†}, Sumanth Devarasetty^a, John Hickey^b, Tarek El-Ashkar^c, Michael Eadon^c, Sanjay Jain^d and Pinaki Sarder^{a*}

^aQuantitative Health Section, Division of Nephrology, Hypertension, and Renal Transplantation, Department of Medicine, University of Florida, Gainesville, FL

^bDepartment of Biomedical Engineering, Duke University, Durham, NC

^cDepartment of Medicine, Division of Nephrology, Indiana University School of Medicine, Indianapolis, IN

^dDivision of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO

[†]Co-First authors

ABSTRACT

Cell types present in a biopsy provide information on disease processes and organ health and are useful in a research setting. Knowledge of cell types, their distributions, and their spatial locations relative to other cells can assist a clinician or researcher in their diagnosis or their investigations. The multiplex imaging technology like co-detection by indexing (CODEX) can provide spatial context for protein expression and detect cell types on a whole slide basis. The CODEX workflow also allows for hematoxylin and eosin (H&E) staining on the same sections used in molecular imaging. In this work, we develop a deep learning pipeline to automatically detect cell types in histologically stained intestine tissue sections by leveraging the ground truth annotations obtained from the corresponding CODEX images. The data consists of 32 paired CODEX and histology samples obtained from four donors where cell composition is determined by multiplexed imaging and single-nucleus RNA and open chromatin assays. We then train a semantic segmentation model on 24 annotated images and holdout 8 samples for testing. In this analysis, we achieved a notable ~4.5X improvement with balanced accuracy over the random baseline for 12 classes. Our work has potential to be used in clinical and basic research to study cell type and cell distributions with functional tissue units to obtain a holistic overview of intestine tissue biology, and the study can be expanded to other organ types as well.

Keywords: Deep learning, semantic segmentation, intestine, histology, CODEX

1. INTRODUCTION

Cell types and their locations in a biopsy provide relevant information on organ health and are useful in research settings. CODEX is a multiplex imaging technology that allows for simultaneous detection of multiple antibody-based targets in tissue samples. This technology adds spatial context to protein expressions with the ability to accurately measure many markers. The abundance and diversity of markers available allows investigators to spatially annotate many cell types and subtypes. In addition, new CODEX workflows enable H&E staining on the same sections used in molecular imaging and allow for robust, reproducible identification and quantification of single cells within complex tissues [1, 2, 3]. CODEX has recently been used to map cell types and proteins in human intestine [2, 4]. In [2], the authors uncover differences in both the composition of functional regions and the molecular identity of the cell states that comprise these anatomical regions. This single cell-level analysis reveals the composition and function of this organ.

* Send correspondence to: Pinaki Sarder, PhD; Email - pinaki.sarder@medicine.ufl.edu

In this work, we present a cell prediction pipeline in intestine with 32 annotated CODEX samples and 12 major cell types with histology pairs. We develop a deep learning-based classification pipeline that learns the complex cellular composition and prevalence from different intestine samples. We train a semantic segmentation network that automatically segments and classifies cell types from histologically stained tissues, with CODEX generated ground truth cell labels [3]. Our approach achieved ~4.5X improvement in balanced accuracy over the random baseline for 12 classes. Our future work will focus on overcoming class imbalance among cell types as well as expanding our training samples to further enhance accuracy.

2. METHODS

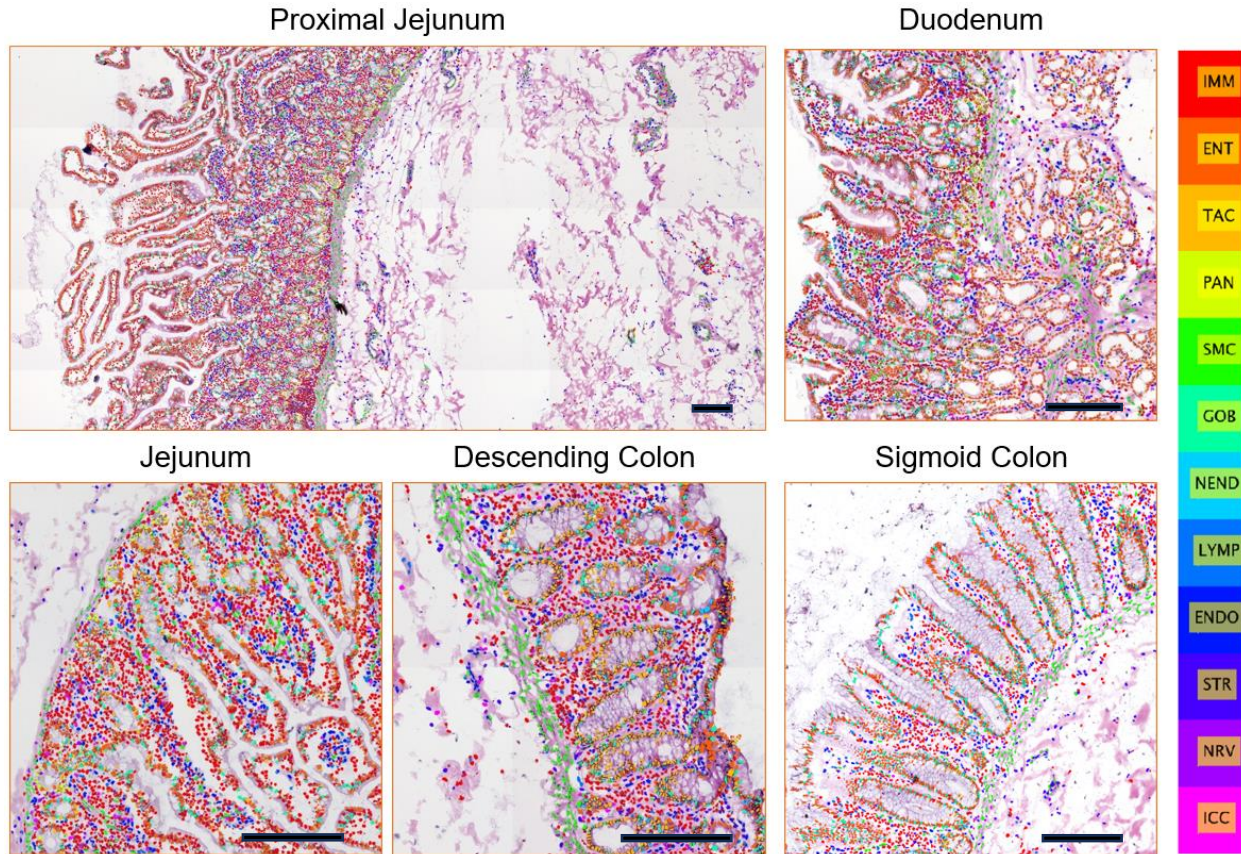


Fig. 1 Cell mapping overlaid on the brightfield histology section shown in different segments of intestine. The cell types in the color bar correspond to IMM:Immune, ENT: Enterocyte, TAC: Transient amplifying cell, PAN: Paneth cell, SMC: Smooth muscle cell, GOB: Goblet cell, NEND: Neuroendocrine cell, LYMP: Lymphatic endothelium, ENDO: Vascular Endothelium, STR: Stromal, NRV: Nerve, ICC: Interstitial cell of cajal. The scale bars correspond to 100 μ m.

Data

The intestine can be partitioned into distinct regions; the duodenum, jejunum and ileum in the small intestine and the ascending, transverse, descending and sigmoid in large intestine. These anatomical segments contain diverse cell types that are both phenotypically and morphologically distinct and spatially organized into different regions across segments. Examples of ground-truth mappings are shown in **Fig. 1** with cell types illustrated in distinct colors.

Cell annotations were initially developed by Hickey *et al.* [2] where spatial identification of 25 cell types at single cell resolution in the intestine samples was performed. Cell types are assigned in the CODEX samples by examining gene expression levels and gene activity scores of known marker genes as well as by labelling the dataset with previously

published scRNA-seq data [5]. For our analysis, the obtained annotations from CODEX samples serve as ground truth labels on the corresponding histology image. We use 32 CODEX whole slide images (WSIs) paired with histology samples obtained from four patients, where each WSI dimensions are of 4536×4704 pixels, in total 21M pixels. Here, we split 24 WSIs from three patients for training and eight holdout WSIs from one patient for testing. Total number of cells mapped to be $>850K$ for training and $\sim 170K$ for testing.

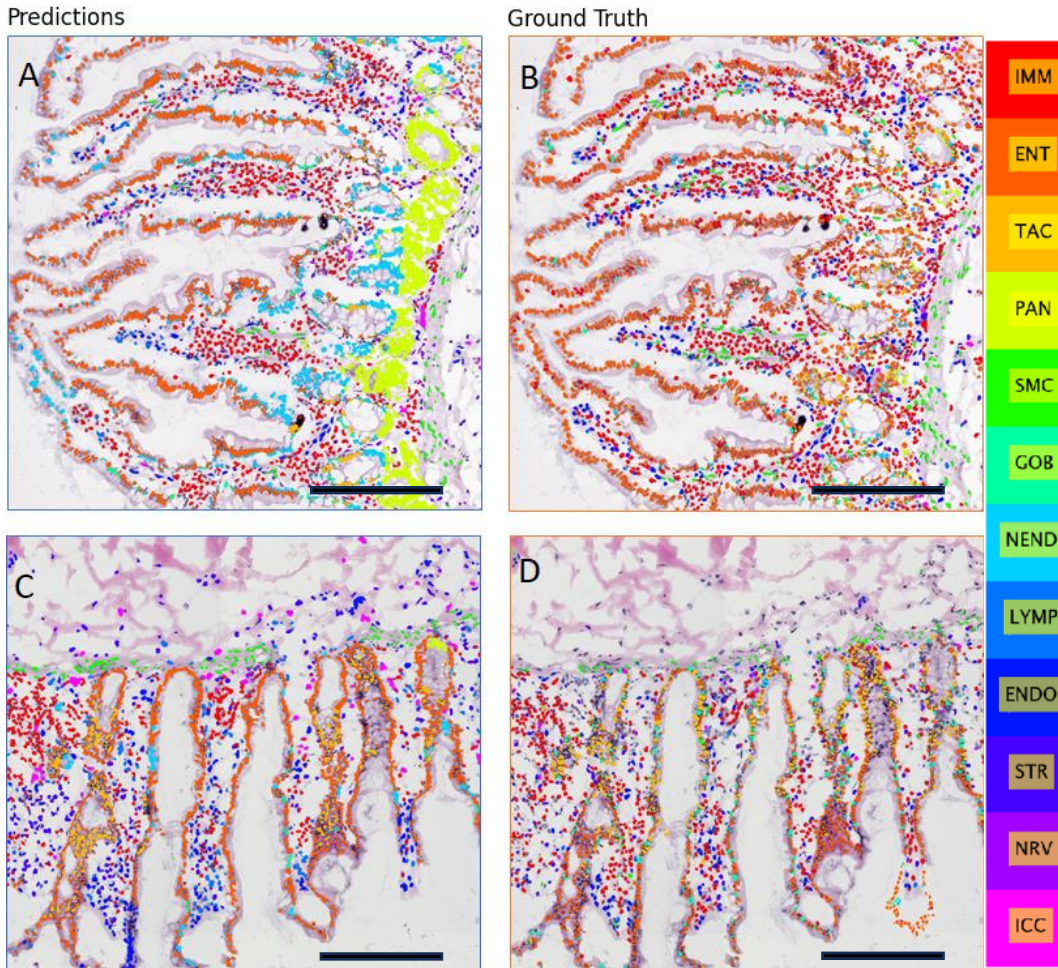


Fig. 2 The ground truth and prediction masks overlaid on the brightfield histology image. (A) & (C) show the model prediction and (B) & (D) the ground truth spatial cell distribution derived from the respective associated CODEX images. The scale bars correspond to 100 μm .

Segmentation

Semantic segmentation of the brightfield WSIs used the Tensorflow Deeplab V3+ framework for training and inference [3,6]. Deeplab V3+ uses atrous convolutional layers to the network to capture contextual information in images at multiple scales. Dilated convolutional layers expand the spatial range of image feature extraction without increasing the number of parameters. The decoder module then up samples the image to create a semantic map of the image. Our network uses the Xception-65 architecture for segmentation. Training was performed using an on-the-fly method of choosing and loading image patches [7]. A network model pretrained on ImageNet was used for transfer learning and fine-tuning. A base learning rate of $1E-4$ was used with an Adam optimizer for categorical cross-entropy loss.

3. RESULTS

The model is trained on the training set to perform the classification as multi-class cell segmentation task. Due to class imbalance in cell types, we use weighted cross entropy loss function where each class (and background) is weighted with

the inverse of their abundance in the training set. Examples of the model prediction (**Fig. 2A** and **2C**) are illustrated in **Fig. 2** along with the corresponding ground truth cell map derived from the corresponding CODEX image (**Fig. 2B** and **2D**). In this analysis, we report multiplicative improvement in balanced accuracy over the random baseline of 8.33% for 12 cell types measured with sensitivity (true positive rate) metric. **Table 1** shows per-class improvement obtained from the trained network predictions on eight WSIs in the test set. On average, we achieved $\sim 4.5X$ improvement in balanced accuracy across all classes in the test images.

Table 1 Classification results of our approach. We report improvement of balanced accuracy over the random baseline of 8.33% for 12 cell class types. The values represent multiplicative accuracy improvement over the baseline per each class for each slide of the test set. The last row is the average performance gain over the baseline per sample across all classes. WSI names are indicated using ID preceded with B012.

Cell Type	B012A_1	B012A_2	B012A_3	B012A_4	B012B_1	B012B_2	B012B_3	B012B_4
Immune	3.28	3.30	3.26	4.43	4.69	4.22	3.32	3.61
Enterocyte	6.00	2.66	1.62	5.60	6.38	2.58	2.87	4.22
Transient amplifying	5.64	6.19	4.65	3.85	5.14	7.14	7.68	2.09
Paneth	6.92	0.00	0.00	9.78	10.28	0.00	0.00	7.67
Smooth muscle	6.80	6.56	7.23	4.63	5.70	3.10	6.49	6.72
Goblet	5.90	5.82	6.23	7.93	4.03	5.60	6.96	9.05
Neuroendocrine	0.65	1.49	0.35	0.31	0.80	0.61	1.83	0.73
Lymphatic endothelium	2.29	3.30	2.00	6.29	4.82	7.88	5.81	3.09
Vascular Endothelium	1.98	4.59	5.33	2.26	5.39	4.80	6.15	5.06
Stromal	4.74	5.83	7.13	4.50	3.62	1.63	4.01	3.93
Nerve	3.45	3.12	5.74	4.33	1.18	0.98	4.62	5.50
Interstitial cell of cajal	3.57	3.97	5.03	6.51	7.28	5.66	5.64	7.66
	4.28	3.91	4.06	5.05	4.96	3.70	4.63	4.96

4. DISCUSSION AND CONCLUSION

In this work, we show the automated segmentation and classification of cell types from brightfield WSIs of frozen intestine biopsies. Spatial location and distribution of specific cell types are important in clinical settings and research studies.

We were able to automatically segment and classify various cell types directly from H&E stained WSIs from intestine. Our initial results demonstrate that the deep learning model effectively learns to identify these cell types. As future work, we plan to address the challenges of class imbalance and develop strategies to further refine the model and enhance classification accuracy.

There are several limitations of our study as well as directions for future work. First, since the ground truths are mostly computationally derived, it is difficult to measure their accuracy and reliability. However, deep learning-based semantic segmentation is commonly robust to slight errors or noise in labels and may even benefit from it. Second, the data size and its heterogeneity are limited as we have a small number of samples from a single institution. In addition, as all samples are from frozen sections where coloring and staining cell types lack differentiation, it is challenging for the deep learning model to discriminate them. Thus, as future work, we plan to expand the training dataset and add FFPE samples with more bright colors.

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